NASA TT F-9074

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FACILITY FORM 602
N71-71413 (ACCESSION NUMBER)
29 (PAGES)
(NASA CR OR TMX OR AD NUMBER)
(THRU)
None (CODE)
(CATEGORY)

Translation of "Das glykolytische Enzymmuster von Leber, Niere, Skeletmuskel, Herzmuskel und Grosshirn bei Ratte und Maus."

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NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON SEPTEMBER 1964

THE GLYCOLYTIC ENZYME PATTERN OF THE LIVER, KIDNEY, SKELETAL AND HEART MUSCLE AND BRAIN IN THE RAT AND MOUSE

R. von Fellenberg, H. Eppenberger,
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The individual stages of glycolysis were elucidated between 1910 and 1940 by Neuberg, von Euler, Embden, Meyerhof, Warburg, Lohmann and the Coris and incorporated in the now validated reaction sequence. The knowledge that glycolysis runs essentially the same course in all animal cells led to the assumption that the enzyme patterns of the individual tissues coincide not only qualitatively but also quantitatively. The biologic function of the enzymes was interpreted as being exclusively catalytic and not at all as regulatory. These reasons account for the absence of comparative investigations of all glycolytic enzymes in various organs. Only in the last few years has the behavior of individual enzymes been investigated under experimental conditions, usually in a pathologic context. We would mention the observations on tumors [1, 5, 19, 22, 40, 41, 57, 59, 60, 64], under the action of hormones [22, 56, 62, 63, 65], especially in diabetes [15, 21, 37, 38, 50] and for imbalanced diet [16-18]. These investigations were almost exclusively confined to the liver.

Abbreviations used. HEX, hexokinase; G-6-P-DH, glucose-6-phosphate dehydrogenase; HP-I, hexose-phosphate isomerase; F-6-P-K, fructose-6-phosphate kinase [or phosphofructokinase]; F-1,6-P-A, fructose-1,6-phosphate aldolase; P-GLY-DH, Glycerine-1-phosphate dehydrogenase; GAP-DH, phosphoglyceraldehyde dehydrogenase; P-GLY-K, phosphoglycerokinase; P-GLY-M, phosphoglyceromutase; ENO, enolase; PYR-K, pyruvate kinase; LDH, lactic dehydrogenase; GOT, glutamate-oxalacetate transaminase; IC-DH, isocitric dehydrogenase; PYL-ASE, phosphorylase; G-6-P-ASE, glucose-6-phosphatase; F-1,6-P-ASE, fructose-1,6-diphosphatase; G, glucose; Pi, inorganic phosphorus; P, ester phosphate; NAD-$NADH_2$, oxidized or reduced nicotinamide-adenine dinucleotide; NADP-$NADPH_2$, oxidized or reduced nicotinamide-adenine dinucleotide phosphate.

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The development and technical refinement of the "optical test" evolved by Warburg and Christian [54] now permits reliable determination of a series of enzymes with relatively little expenditure of time or material. It is thereby possible to compare enzyme determinations made in different laboratories. Bücher et al. were the first to compare the enzyme pattern not only in a pathologic setting but also from the standpoint of extreme functional contradictions [66]. Their investigations were chiefly concerned with the enzymes of anaerobic glucose metabolism which are localized in the C-compartment (cytoplasmic compartment) of the cell, defined by Bücher and Klingenberg [6]. /335

The present study compares the glycolytic enzyme patterns of the C-compartment of some mouse and rat organs. An attempt is made to determine quantitative organ differences and to relate them to the known organ-specific features of energy metabolism.

Methods

1. Animal material. The mice were from a Swiss albino inbred strain reared in our institute. The rats were of an albino strain of the Nestle Co. [1].* Only male animals were used. The weight of the rats varied between 230 and 370 g, that of the mice between 25 and 32 g. The test animals were fed on a mixed diet and received water ad lib.

2. Preparation of homogenates. The animals were decapitated and exsanguinated. The organs were removed at once, rinsed with spring water for a short while, dried with filter paper and placed in ice-cooled dishes. The media used were at first TAEB (triethanolamine-HCl-EDTA buffer, 50 mM triethanolamine, 5 mM EDTA, pH 7.5), later, sucrose solution (0.25 M sucrose, 5 mM EDTA). With use of TAEB the methods employed by us for protein determinations gave false positive results. Comparative determinations with both media showed no important differences in the enzyme activities.

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*We thank Fräulein Dr. J. Antener of Nestle in Vevey for kindly making available the animals.

The organ specimens were homogenized for 30 sec - 1 min with nine parts of medium in ice-cooled glass homogenizers, by the Potter-Elyehjem technique. The homogenates in an amount of 0.4 ml were centrifuged in plastic tubes for 5 min at 8000 g (15,000 rpm) in a Spinco microcentrifuge at room temperature (Beckman Spinco microfuge model 152). All the enzymes were estimated in the homogenate supernatant. This corresponds to the C-compartment as defined by Bücher [6]. The supernatants obtained were frozen in a Dewar vessel with dry ice until the time of determination of enzyme activities.

3. Methods of determination*. HEX, G-6-P-DH, HP-I, F-6-P-K, F-1, 6-P-A, P-GLY-DH, GAP-DH, P-GLY-K, P-GLY-M, ENO, PYR-K, LDH, GOT, IC-DH were determined by the "optical test". The details are summarized in the appendix. Most of the enzymes were measured in the equilibrium direction at which the maximum substrate turnover occurred (exception: F-1,6-P-A). The measuring instrument was an Eppendorf photometer (filter 366 mμ) with a WW linear direct recorder (firm Dr. A. Vaudaux, Binningen, Basle). For G-6-P-ASE the /336
increase in free glucose was determined by the glucose-oxidase method. PYL-ASE and F-1,6-P-ASE were determined by measuring the released inorganic phosphorus (Pi) [64]. All the measurements were at pH 7.5 and a temperature of 25°C.

Apart from G-6-P-ASE all enzymes gave a zero order reaction. The structure-bound (microsomes) G-6-P-ASE showed an exponential course [28]. Since for the short incubation period of 5 min chosen by us the conditions were approximately linear, the values were not corrected for the deviating kinetics. The supernatants for analyses were diluted with distilled water. The enzyme unit chosen was μM/min/g fresh weight, or μM/min/mg protein [46].

4. Protein determinations. Protein was determined by the method of Folin and Ciocalteu as modified by Lowry [39]. Bovine serum albumin served as standard. The phenol reagent of the firm Merck (Art. No. 9001) was diluted 1:4.

5. Statistical data:

$$\bar{x} = \text{mean}$$

$$\pm s = \text{standard deviation} = \sqrt{\frac{(\bar{x} - x)^2}{N - 1}}$$

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*For abbreviations see footnote on p. 1.

$$CE = \text{coefficient of error} = \frac{s}{\bar{x}} \cdot 100$$

Results

1. General observations. In the first series of tests we determined the scatter of the enzyme analyses due to technical factors. For this purpose a rat homogenate supernatant was frozen in small portions. This material was used to make one enzyme determination a day on 8 different days, with different solutions and in part by different persons. The results of this experiment are listed in Table 1. The coefficient of error was used as a relative measure of scatter. Comparison of the values obtained with that in Tables 2 and 3 shows that the observed scatter was for most enzymed and organs in the same order of magnitude as the technical scatter.

TABLE 1

Scatter of the Method
Unit: µM/min/g fresh weight

Enzymes	LDH	GOT	F-1-6-P-A	G-6-P-DH	IC-DH	F-6-P-K	P-GLY-DH	GAP-DH	ENO	HEX	PYR-K	HPI
$\bar{x}$	35.7	27.8	2.9	0.9	8.8	2.7	7.3	43.7	6.3	32.2	27.1	47.8
±s	16.5	7.0	1.3	0.2	2.3	1.3	3.9	22.8	2.4	7.9	5.2	6.9
Fresh weight	46.2	25.2	44.8	22.2	26.1	48.1	53.4	52.2	38.1	24.5	19.2	14.4

The causes of the relatively high technical variability are manifold, the most important factor being the differences in purity of the

enzymes and coenzymes. We would particularly stress the relatively poor keeping qualities of DPN-H solutions although discoloration cannot here be taken as indicating unsuitability for use.

Tables 2 and 3 group the activities of the glycolytic enzymes of the C-compartment in the mouse and rat. In addition, G-6-P-DH was measured as the limiting enzyme of glucose oxidation and IC-DH as a supplementary TPN-dependent enzyme. The results are given per gram fresh weight. Table 4 gives the protein values for the five organs of the rat and mouse. If instead of the fresh weight we take soluble cytoplasmatic protein as a reference magnitude for the enzyme activities, we get even greater differences for the various enzymes between organs.

2. Comparison of the results with values in the literature. Bücher et al. [13] have compared the results of various workers for some glycolytic enzymes. They found that the values may differ from author to author by powers of ten. This is due partly to differences in the experimental arrangements and partly to differences in preparing the test material. In this situation values which agree within the same order of magnitude may be regarded as satisfactory. Comparison of our results with the values in the literature gives the following picture: /338

HEX: In the rat heart our values are close to those of Lamprecht [13]. For the liver Trautschold [13] reports values some three times higher. Comparison with the investigations of Long [1] gives good agreement for muscle and heart, values for the liver nine times higher in our work and activities in the brain six times higher in the work of Long.

G-6-P-ASE: The exponential course of the kinetics means that the measured activity is dependent on incubation time. Since most investigators [10, 18, 38, 59] incubate for 30-60 min while we did so only for 5 min, our results are, as would be expected, somewhat higher. /339

G-6-P-DH: Both the data of Glock and McLean [13, 19] and those of Bücher [13] are close to our results.

GAP-DH: The values of Bücher [13] for all organs are about double our own.

F-1,6-P-A: For this enzyme the results of all investigators are in good agreement [13, 17, 35, 52].

TABLE 2

Enzymes of the C-compartment in the rat Unit: μM/min/g fresh weight

Organ / Enzyme	Liver		Kidneys		Muscle		Heart		Brain	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	±s	$\bar{x}$	±s
PYL-ASE	21.0	1.3	-	-	180.0	57.2	58.0	9.3	-	-
HEX	27.1	5.3	36.3	11.9	19.9	3.3	59.0	22.5	10.7	3.8
G-6-P-ASE	8.7	2.7	4.4	1.2	-	-	-	-	-	-
HP-I	16.0	7.4	20.0	9.7	31.0	15.4	18.0	8.2	13.0	7.4
P-6-P-K	2.7	1.9	1.2	0.3	1.7	0.7	1.6	0.9	1.1	0.2
F-1,6-P-ASE	4.1	1.3	2.0	0.6	-	-	-	-	-	-
F-1,6-P-ALD	5.2	100	7.0	1.3	41.0	4.9	16.0	1.0	7.1	2.1
GAP-DH	70.2	24.8	82.5	25.5	347.0	127.0	113.0	30.9	41.7	16.5

[Table 2 continued next page]

[Table 2 continued]

Organ / Enzyme	Liver		Kidneys		Muscle		Heart		Brain	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	x	$\pm s$	$\bar{x}$	$\pm s$
P-GLY-K	30.0	12.2	43.0	5.1	122.0	8.7	52.0	3.6	26.0	3.5
P-GLY-M	52.0	14.4	56.0	15.7	242.0	96.6	95.0	43.5	35.0	5.2
ENO	6.6	3.3	10.3	2.2	78.3	2.7	12.1	6.2	3.9	1.2
PYR-K	55.5	14.7	19.2	2.1	216.4	43.9	58.4	12.9	74.8	48.3
LDH	658.0	212.0	71.0	10.0	384.0	108.0	214.0	78.0	57.0	43.0
P-GLY-DH	19.0	1.9	7.0	3.5	9.0	6.4	2.8	2.0	1.1	0.2
G-6-P-DH	0.5	0.02	1.2	0.3	-	-	0.3	0.01	0.8	0.3
IC-DH	8.8	3.2	10.7	0.8	1.2	0.55	22.4	6.3	-	-
GOT	36.3	13.0	29.7	6.6	26.5	1.6	85.1	19.2	14.8	6.6

TABLE 3

Proteins for rat and mouse organs
(mg soluble protein/g fresh weight)

Organ	Liver		Kidneys		Muscle		Heart		Brain	
Enzyme	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s
HEX	16.6	8.8	17.6	9.6	17.1	9.1	55.6	27.4	7.6	4.7
G-6-P-ASE	6.6	2.1	2.9	2.4	-	-	-	-	-	-
HP-I	20.1	5.1	23.7	10.5	32.5	10.9	30.3	6.1	19.2	4.8
P-6-P-K	2.1	0.7	1.6	0.8	1.7	0.54	1.2	0.4	1.0	0.2
F-1,6-P-ASE	2.9	1.6	2.4	1.6	-	-	-	-	-	-
F-1,6-P-ALD	4.1	1.2	6.6	3.3	29.0	12.0	11.0	3.2	7.1	1.3
GAP-DH	70.0	12.9	50.0	6.0	166.0	81.8	74.0	19.3	36.0	6.3

[Table 3 continued next page]

[Table 3 continued]

Organ / Enzyme	Liver		Kidneys		Muscle		Heart		Brain	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
P-GLY-K	82.0	13.0	82.0	9.5	103.0	15.4	46.0	4.1	28.0	3.3
P-GLY-M	34.0	2.9	32.0	9.8	145.0	26.0	48.0	14.4	32.0	10.5
ENO	4.4	1.8	6.0	5.9	52.9	3.2	19.0	9.3	7.2	4.8
PYR-K	20.1	18.9	16.7	7.9	107.4	19.4	55.0	10.8	48.8	9.5
LDH	405.8	281.3	109.4	39.9	277.7	45.3	132.5	63.0	38.5	14.1
P-GLY-DH	10.0	2.6	4.0	1.9	4.0	2.3	1.7	0.87	1.2	0.6
G-6-P-DH	0.2	0.1	7.8	0.6	0.14	0.01	0.27	0.06	0.6	0.14
IC-DH	8.9	2.7	9.5	4.7	0.7	0.25	12.7	2.2	0.5	0.1
GOT	27.8	17.4	16.3	6.8	14.5	3.6	52.1	31.2	14.9	2.7

P-GLY-DH: In the liver our values concur with those of Boxer and Shonk [5]. Bücher's [13] values are five times higher. The reason for this discrepancy, despite similar experimental conditions, could not be established. The key position of this enzyme for the Bücher cycle [6, 66] has led investigators to look for a change in the LDH/G-DH ratio in tumors. However, as long as such great discrepancies prevail both in G-DH and LDH we do not feel that this ratio can provide any information.

TABLE 4

Protein Values for Rat and Mouse Organs
(mg soluble protein/g fresh weight)

Rat		Liver	Kidneys	Muscle	Heart	Brain
Rat:	$\bar{x}$	112	103	50	59	43
	± s	32	27	15	30	16
Mouse:	$\bar{x}$	127	83	45	80	53
	± s	35	27	26	34	12

LDH: The findings for this enzyme show the greatest range of scatter. For the liver the values lie between 80 [13] and 650 µM/min/g fresh weight in our case. For brain and muscle we obtained similar results to those of Bücher [13]. As we showed in another paper, these differences must be attributed to the existence of several isoenzymes with different optimal conditions for detection and showing different distribution patterns in different organs [47].

These comparisons show that considerable discrepancies still prevail for most enzymes. Nevertheless, it is now possible, independent of the laboratory, to obtain results which lie within the same order of magnitude. Improved and purer enzyme preparations should in the next few years make it possible to express the enzyme patterns of different tissues with the same precision now possible for some metabolites.

3. Critical appraisal of investigations: Three objections must be expected to the experimental arrangement used by us. The definition of the C-compartment implies that from the complex structure of the cell one part has been arbitrarily singled out which only crudely corresponds to the intracellular structure. In doing so, both the submicroscopic structure of the cytoplasm and the interactions between the morphologic units of the cells are disregarded. Thus, G-6-P-ASE [28] and possibly also HEX are structurally-bound (endoplasmic reticulum) in the C-compartment and cannot therefore be equated with the other, free enzymes of the C-compartment. Less weighty is the objection that the C-compartment was analyzed divorced from other organelles, since it is clear that the mitochondrial membrane is impermeable for $NADH_2$ and $NADPH_2$. As Bücher /340

et al. have shown, there is, in fact, a good relation between the enzymes of the C-compartment, on the one hand, and the, probably intravital, metabolite concentrations.

Measurement of the enzyme activities of whole organs makes no allowance for the very heterogeneous histologic structure of these organs. The enzyme activities may greatly vary in different types of cell and distort the enzyme pattern of the whole organ.

Along with this morphologic objection a biochemical one may also be raised. Our measurements of enzyme activities relate to the sum of the catalytic properties of a tissue for a certain substrate. Recent investigations have shown that at least LDH, IC-DH and G-6-P-DH occur in different molecular species -- isoenzymes -- distinguished inter alia by different substrate optima. Consequently, the result of a determination will depend, on the one hand, on the system conditions and, on the other, on the isoenzymes present. We have shown elsewhere [47] that LDH analyses give results for many organs as much as 50 per cent too low. Only prior separation into individual isoenzymes and separate determination under given optimal conditions yields the actual maximal LDH activity. All these factors indicate that for comparative enzyme analyses concerned with total activity, differences should be taken as real only if they exceed one decimal power.

Discussion

1. Organ specificities in the glycolytic enzyme pattern. The energy-metabolism of the animal cell has essentially the same course despite the extreme morphologic and functional differentiation of the individual cell types [42].

The abnormal behavior of tumors has led many workers to look for specific changes in energy metabolism in these cells. Although many quantitative differences from normal cells were found, qualitatively the tumor cells never behaved any differently from normal cells [24]. The countless separate analyses of tumor metabolism in relation to normal tissues do not, however, give an idea of the course of the entire metabolic chain.

The results in Figs. 1 and 2 have been grouped in condensed form for comparison of the glycolytic enzyme pattern of the different organs. As they show, considerable differences exist between the different organs. The enzyme patterns may be characterized in two ways. /341

Qualitative differences. Certain enzymes are completely absent, i.e., cannot be detected by the technique used by us. Their activity for the enzymes detected in the "optical test" was under 0.2 μM/min/g fresh weight and for the other enzymes under 1.0 μM/min/g fresh weight.

Quantitative differences. For reasons given above a difference of at least one order of magnitude must be insisted upon.

Taking into account both aspects the enzyme patterns may be divided into two types: on the one hand, organs (liver, kidney) which have active F-1,6-P-ASE and G-6-P-ASE (production of G-6-P from lactate), and, on the other, organs (skeletal muscle, heart muscle, brain) which have no phosphate-ester hydrolases (exclusive production of lactate from G-6-P).

The brain takes a special place in that in this organ some of the glycolytic enzymes occur within the mitochondria. Our analyses of the C-compartment cannot therefore be taken as representative of the total glycolysis of this organ [34].

The first group of organs is enzymatically characterized by the presence of relatively large amounts of F-1,6-P-ASE and G-6-P-ASE. The liver and kidney are the only organs which can release glucose into the bloodstream and hence they play a decisive part in regulation of the blood sugar level. The liver is the scene of considerable PYL-ASE activity, while this enzyme could not be detected in the kidney by our methods. However, it must be mentioned that the kidney glycogen is present chiefly in the cells of the proximal tubules; even considerable PYL-ASE activities of these cells may be missed when examining the total organ.

In the second group of organs, of the muscle type, G-6-P-ASE and F-1,6-P-ASE are absent. As has been known for years, glycolytic energy production occupies the foreground in these organs during work. Liver tumors behave in this respect like the musculature.

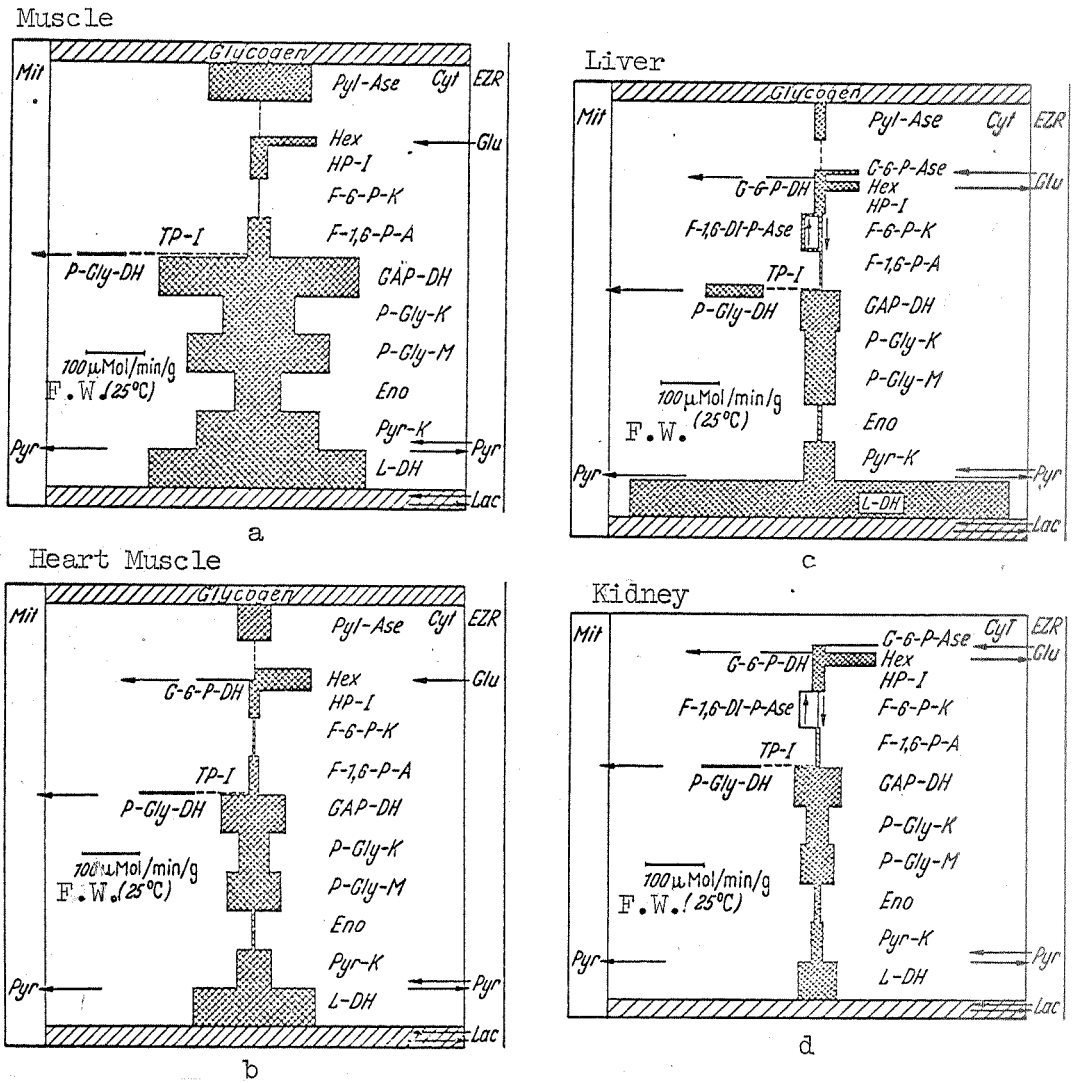

Fig. 1

a) Enzyme pattern for skeletal muscle; b) for heart muscle; c) for the liver and d) for the kidney.

Mit (mitochondrium), Cyt (cytoplasm), EZR (extracellular compartment)

Striking quantitative differences are shown by PYL-ASE and the enzymes between F-1,6-P-ALD and LDH. The activities of these enzymes are much higher in the muscle than in the liver and kidney. In contrast, the amounts of the enzymes between HEX and F-6-P-K are about the same in all organs. /342

2. Enzyme patterns and aerobic metabolism. In Table 5, as a measure of aerobic metabolism, pyruvate oxidation of rat organ slices and the oxygen uptake of the organs in vivo are compared with the GAP-DH activity of the individual organs.

We chose GAP-DH as representative of enzymes between F-1,6-P-ALD and LDH since the ratio of these enzymes in the individual organs is about the same. GAP-DH activity is a reciprocal of the oxygen uptake, pyruvate oxidation and blood supply.

TABLE 5

	Liver	Kidney	Muscle	Heart	Brain
QO_2 (pyruvate) Slices	16.5	30.2	5.9	15.4	11.1
O_2 consumption μM/min/g fresh weight		2.22	0.12	4.32	1.47
Blood flow ml/min/g fresh weight		3.61	0.04	0.84	0.54
GAP-DH activity μM/min/g fresh weight	70.0	83.0	347.0	113.0	42.0

The values for the O_2 consumption and blood flow are in vivo data for humans [49].

3. "Large" and "small" enzymes. The great differences in activity of the individual glycolytic enzymes are striking. Thus, in skeletal muscle, F-6-P-K at 2 μM/min/g fresh weight co-exists with LDH at 380 μM/min/g fresh weight. Bücher [66] therefore speaks of "large" and "small" enzymes. This division is obviously not only of heuristic importance. Thus, in relation to the "small" enzymes (HEX, HP-I, F-6-P-K) hardly any organ differences are discernible. In contrast, the "large" enzymes (GAP-DH, P-GLY-K, P-GLY-M, ENO, PYR-K, LDH) show significant differences. An exception is F-1,6-P-ALD which, though it must be included among "small" enzymes, shows considerable organ differences. The great differences in the total activities of the glycolytic enzymes and the absence of organ differences for the "small" enzymes raises the question as to whether the substrate flow may possibly be limited by certain enzymes [49]. Elucidation of this problem calls for an analytical description of the most important control factors in glycolysis. /343

4. Glycolysis. The breakdown of glucose to lactate consists essentially of two irreversible phosphorylation reactions (G → G-6-P, F-6-P → F-1,6-P) alternating with two equilibrium systems (G-6-P ⇌ ⇌ G-1-P ⇌ F-1-P and F-1,6-P ⇌ lactate). The glycolytic system is in a steady state, i.e., constant amounts of substrate are turned over per unit time. The individual reactions are here more or less in thermodynamic imbalance determined by the preceding and succeeding reactions.

Glucose and F-6-P are both phosphorylated via ATP involving a considerable fall in free energy. For thermodynamic reasons both these stages are irreversible, having regard to the conditions in the cell. Hydrolysis of G-6-P and F-1,6-P thus occurs via special enzyme systems with formation of inorganic phosphate. For the irreversible stages the substrate flow may be modified by the educts but not by the products. /344

5. Feedback. GLY-P-K and PYR-K each reversibly transfer a phosphate residue from an energy-rich compound to ADP with formation of ATP. Both reactions are closely linked with the $NAD/NADH_2$ system. For the first ATP formation NAD is reduced in the preliminary GAP-DH reaction, in the second ATP synthesis DPNH is oxidized by the subsequent LDH reaction. The reactions of LDH and GAP-DH system are thus genuine feedbacks. The P-GLY-DH reaction, which in the mammalian cell initiates a secondary path of glycolysis, is the third system importantly involved in $NAD/NADH_2$ turnover and hence, may come into competition with the LDH system [5, 6]. For the ATP/ADP system there is no feedback in the strict sense. Although both ATP-forming reactions

have a remote effect on ATP-consuming processes, both ATP and ADP have only a promoting but not a controlling effect, since both phosphorylation reactions are irreversible.

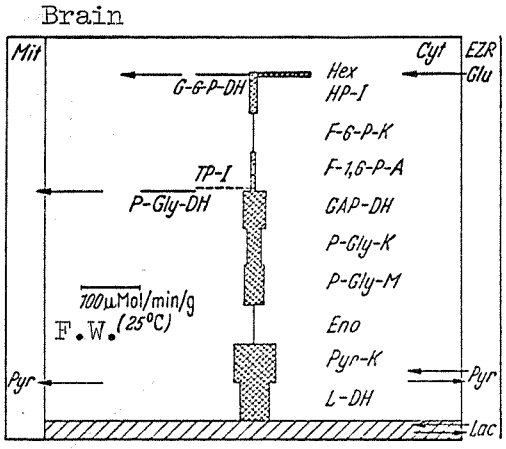

Fig. 2

Enzyme pattern for brain.

6. Anoxia. Huckabee [30] has shown that in vivo under aerobic conditions the lactate/pyruvate ratio is constant. On incurring an O_2 deficit even if small [31-33] the ratio shifts and excess lactate forms. This shift must be attributed to the change in the $NAD/NADH_2$ ratio in favor of LDH. The increased formation of NAD by the LDH system is directly proportional to the O_2 deficit. Through feedback the increased NAD yield accelerates the GAP-DH reaction and promotes irreversible phosphorylation through the resulting formation of ATP. The "steady state" shifts in the sense of increased glucose degradation. /345

7. Enzymes as control factors. The result of depletion of glucose supply is that the flow system passes from the "steady state" to the

status of thermodynamic equilibria of the individual reactions. Since in thermodynamic equilibrium no change in free energy occurs, this, in anaerobic conditions, means death of the cell.

The primary function of the enzymes is their catalytic action. This depends both on the enzyme concentration (maximum activity) and on the kinetic properties of the enzymes. If all the enzymes are present in excess over their substrates they cannot exercise any controlling function in the flow system. In this case the substrate yield and feedback mechanisms alone determine the "steady state." However, it is possible that certain enzymes have only a limited catalytic action in relation to their substrates, i.e., they limit substrate turnover. In this case, the enzymes have a controlling function, i.e., they help to maintain the "steady state" and counteract cell depletion. It is still not known how far glycolysis is limited by enzymes. Only study of the enzyme activities together with the enzyme kinetics of all the enzymes involved can provide further information on this.

METHODOLOGICAL APPENDIX ON THE "OPTICAL TEST"

1. The substances used for the "optical test" were stored as stock solutions. Immediately prior to the measurements, two working solutions were prepared:

Solution 1. Containing coenzymes, cofactors and enzymes (for coupled test).

Solution 2. Containing the substrate for incubation.

2. The end volume of the system was 0.7 ml. The procedure was as follows:

Solution 1. 0.5 ml Analysis. 0.1 ml

Preliminary incubation until no further change in extinction.

Solution 2. 0.1 ml /346

Start: the reaction was allowed to run 1-10 min depending on the reaction rate.

3. Working solutions for the enzyme reactions. The numerals given against Solutions 1 and 2 refer to the number of the stock solutions in 4.

Enzyme	Solution 1	Solution 2	Literature reference
L-DH	1 T 29	1 T 10	F. Kubowitz, P. Ott
	19 T Buffer	4 T Buffer	Biochem.Z. 314, 94 [43]
GPT	1 T 29	1 T 13	A. Karmen
	1 T 12	4 T Buffer	J. Clin. Invest. 34, 131 [55]
	1 T 21		
	17 T Buffer		

[Table cont'd. on next page]

[Table cont'd.]

Enzyme	Solution 1	Solution 2	Literature reference
GOT	1 T 29	1 T 13	A. Karmen
	1 T 7	4 T Buffer	J. Clin. Invest. 34, 131 [55]
	1 T 28		
	17 T Buffer		
F-1,6-P-A	1 T 29	1 T 8	E. Racker
	1 T 24	4 T Buffer	J. Biol. Chem. 167, 843 [47]
	18 T Buffer		
G-6-P-DH	1 T 30	1 T 9	O. Warburg, W. Christian
	1 T 33	4 T Buffer	Biochem. Z. 287, 291 [36]
	18 T Buffer		
IC-DH	1 T 30	1 T 6	S. Ochoa
	1 T 33	4 T Buffer	J. Biol. Chem. 174, 133 [48]
	18 T Buffer		
GLY-P-DH	1 T 29	1 T 19	T. Baranowski
	19 T Buffer	4 T Buffer	J. Biol. Chem. 180, 535 [49]
GAP-DH	2 T 29	1 T 17	G. Beisenherz et al.
	1 T 33	4 T Buffer	Z. Naturforsch. 8b, 555 [53]
	1 T 32		
	1 T 23		
	1 T 18		

[Table cont'd. on next page]

[Table cont'd.]

Enzyme	Solution 1	Solution 2	Literature reference
GAP-DH	14 T Buffer		
ENO	1 T 29	1 T 1	Th. Bücher
	1 T 33	4 T Buffer	Methods in Enzymol. 1, 427 [55]
	1 T 34		
	1 T 31		
	1 T 20		
	1 T 21		
	14 T Buffer		
PYR-K	1 T 29	1 T 3	G. Beisenherz et al.
	1 T 31	4 T Buffer	Z. Naturforsch. 8b, 555 [53]
	1 T 21		
	1 T 33		
	1 T 34		
	15 T Buffer		
HEX	1 T 29	1 T 5	E. Schmidt, F. W. Schmidt
	1 T 3	1 T 29	E. Wildhirt
	1 T 32	3 T Buffer	Klin. Wschr. 37, 1221 [59]
	1 T 33		
	1 T 34		
	1 T 20		

[Table cont'd. on next page]

[Table cont'd.]

Enzyme	Solution 1	Solution 2	Literature reference
HEX	1 T 21		
	13 T Buffer		
HP-I	1 T 30	1 T 16	M. W. Slein
	1 T 33	4 T Buffer	Methods in Enzymol., 1, 364 [55]
	1 T 22		
	17 T Buffer		
FP-K	1 T 29	1 T 16	E. Racker
	1 T 32	4 T Buffer	J. Biol. Chem. 167, 843 [47]
	1 T 25		
	1 T 24		
	0.5 T 33		
	15.5 T Buffer		
P-GLY-M	1 T 29	1 T 17	V. W. Rodwell et al.
	1 T 31	4 T Buffer	Biochim. Biophys. Acta (Amst.) 20, 395 [56]
	1 T 33		
	1 T 34		
	1 T 20		
	1 T 21		
	1 T 26		
	13 T Buffer		

[Table cont'd. on next page]

[Table cont'd.]

Enzyme	Solution 1	Solution 2	Literature reference
P-GLY-K	2 T 29 1 T 33 1 T 32 1 T 27 1 T 18 14 T Buffer	1 T 17 4 T Buffer	

4. Stock solutions. /347

a) Substrates

1. Boehringer 2-phosphoglyceric acid 10 mM;
2. Boehringer 6-phosphogluconate 15 mM;
3. Boehringer phosphoenolpyruvic acid 30 mM;
4. Laevosan fructose 2220 mM;
5. Fluka L-malate 60 mM;
6. Fluka isocitrate 200 mM;
7. Biochemica Roche aspartate 200 mM;
8. Boehringer fructose-1,6-diphosphate 30 mM;
9. Boehringer glucose-6-phosphate 30 mM;
10. Boehringer pyruvate 6mM;
11. Boehringer oxalacetate 6 mM;

12. Biochemica Roche L-alanine 200 mM;

13. Biochemica Roche α-ketoglutarata 200 mM;

14. Boehringer glucose-1-phosphate 100 mM;

15. glucose 1000 mM;

16. Boehringer fructose-6-phosphate 12 mM;

17. Boehringer 3-phosphoglyceric acid 300 mM;

18. Glutathionate 300 mM Boehringer;

19. Calbiochem dihydroxyacetone phosphate 30 mM.

b) Enzymes

20. Boehringer PYR-K 0.2 mg/ml;

21. Boehringer LDH 0.5 mg/ml;

22. Boehringer G-6-P-DH 0.1 mg/ml;

23. Boehringer 3-P-GDY-K 2 mg/ml;

24. Boehringer GLY-P-DH/TIM 0.2 mg/ml;

25. Boehringer F-1,6-P-A 0.2 mg/ml;

26. Boehringer ENO 0.2 mg/ml;

27. Boehringer GAP-DH 0.2 mg/ml;

28. Boehringer M-DH 0.5 mg/ml;

c) Coenzymes

29. Boehringer $NADH_2$ 3mM;

30. Boehringer NADP 6 mM;

31. Boehringer ADP 30 mM;

32. Boehringer ATP 60 mM;

d) Cofactors

33. $MgSO_4$ 200 mM;

34. KCl 2000 mM.

Summary

1. We have measured the activities of the glycolytic enzymes in the cytoplasm of various organs (liver, kidneys, skeletal and heart muscle and brain) of the rat and mouse. /348

2. For each organ of the rat an enzyme distribution pattern was established and is discussed.

3. Possible restriction of substrate flow is discussed on the basis of the very low activities of certain enzymes.

4. In this connection the intracellular control mechanisms of glycolysis are discussed.

Received: 19 June 1962

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